52. The non-human living organism of claim 50, wherein the organism is a yeast.

REMARKS

Claims 25, 47, and 48 have been canceled, without prejudice or disclaimer thereof, claims 5-8, 14-15, 18, 20, 21, 24, 26-46 and 49-52 have been amended to delete multiple dependencies. Because the foregoing amendments do not introduce new matter, entry thereof by the Examiner is respectfully requested.

Applicants respectfully request formal examination of this application in view of the above amendments. This application is now in condition for allowance and early notice to that effect is respectfully solicited.

Should the Examiner have any questions or comments regarding the pending application or this Preliminary Amendment, the Examiner is requested to call the undersigned at the number below.

If there are any fees due in connection with the filing of this Preliminary Amendment, please charge the fees to our Deposit Account No. 19-0741.

Respectfully submitted,

July 31, 2001
Date

for Stephen B. Maebius
Registration No. 35,264

Reg. No. 38,819

FOLEY & LARDNER
3000 K Street, N.W., Suite 500
Washington, D. C. 20007-5109
Telephone: (202) 672-5569
Facsimile: (202) 672-5399

PATENT & TRADEMARK OFFICE JUL 31 2001 TC1817

Version with Markings to Show Changes Made

5. (Amended) The DNA molecule according to [claims 1 to 4] claim 1, wherein sequences A and B are in an opposite direction.

6. (Amended) The DNA molecule according to [claims 1 to 5] claim 1, wherein the site specific recombinase [specific of said] targeting sequence SSRTS L1 and the site specific recombinase [specific of said] targeting sequence SSRTS L2 are the same.

7. (Amended) The DNA molecule according to [claims 1 to 5] claim 1, wherein the site specific recombinase [specific of said] targeting sequence SSRTS L1 and the site specific recombinase [specific of said] targeting sequence SSRTS L2 are different.

8. (Amended) The DNA molecule according to [claims 1 to 7] claim 1, wherein the recombinase [specific of said SSRTS is] targeting sequences are selected from the group of site-specific recombinases composed of the Cre recombinase of bacteriophage P1, the FLP recombinase of Saccharomyces cerevisiae, the R recombinase of Zygosaccharomyces rouxii pSR1, the A recombinase of Kluyveromyces drosophilarium pKD1, the A recombinase of Kluyveromyces waltii pKW1, the integrase λ Int, the recombinase of the GIN recombination system of the Mu phage, of the bacterial β recombinase or a variant thereof.

14. (Amended) The DNA molecule according to [claims 1 to 13] claim 1, wherein said DNA molecule is further flanked by at least site specific recombinase targeting sequences (SSRTS).

15. (Amended) The DNA molecule according to [claims 1 to 14] claim 1, wherein said sequences A and B are selected in the group consisting of non transcribed sequence, transcribed but not translated sequence, transcribed and translated sequence.

18. (Amended) The DNA molecule according to [claims] claim 16 [and 17], wherein sequences A and/or B are coding for at least one exon, or a fragment thereof.

20. (Amended) The DNA molecule according to [claims] claim 16 [and 17], wherein said protein is encoded by a cDNA sequence, and wherein an IRES sequence is inserted 5', or 3', or 5' and 3' to said cDNA sequence.

21. (Amended) The DNA molecule according to [claims] claim 17 [to 20], wherein said reporter protein is selected in the group consisting of autofluorescent proteins and enzymes detectable by a histochemical process.

24. (Amended) A vector [Vector] comprising the isolated DNA molecule of [claims 1 to 23] claim 1.

26. (Amended) An isolated [Isolated] transgenic host cell transformed by an isolated DNA molecule according to claim [claims] 1 [to 23 or a vector according to claim 24].

27. (Amended) The isolated [Isolated] transgenic host cell according to claim 26 wherein sequences of homology are present at both extremities of said DNA molecule.

28. (Amended) The isolated [Isolated] transgenic host cell according to claim 27 wherein said isolated DNA molecule or said vector is integrated by homologous recombination in at least one targeted locus of the genome of said cell.

29. (Amended) The isolated [Isolated] transgenic host cell according to claim 26 wherein said isolated DNA molecule or said vector is integrated in sites of the genome chosen among polyA sites and gene promoters.

30. (Amended) The isolated [Isolated] transgenic host cell according to claim 26 wherein said isolated DNA molecule or said vector is randomly integrated in at least one locus of the genome of said cell.

31. (Amended) The isolated [Isolated] transgenic host cell according to claim 26 wherein said isolated DNA molecule or said vector is maintained in said cell in an episomal form.

32. (Amended) A transgenic non-human [Transgenic] organism[, excepted humans,] comprising at least one cell according to claim [claims] 26 [to 31].

33. (Amended) A method [Method] for the stable inversion of a DNA sequence comprising the steps of:

(i) contacting a DNA molecule according to [claims] claim 1 [to 23, or a DNA vector according to claim 24] with at least one site specific recombinase [specific of said] targeting sequence SSRTS L1 and one recombinase [specific specific of said] targeting sequence SSRTS L2;

(ii) inversion of said sequences A and B or sequence A or sequence B by recombination catalyzed by said recombinase at either SSRTS L1 or L2 sequences; and

(iii) excision by recombination catalyzed by said recombinase of a DNA fragment comprised in between the SSRTS L1 or L2 sequences that are now present in direct orientation following the inversion of step (ii), and that are able to recombine with one another.

34. (Amended) The method [Method] according to claim 33 wherein said DNA fragment excised in step (iii) comprises the sequence A.

35. (Amended) A method [Method] for obtaining a transgenic cell of which at least one allele of a DNA sequence of interest is invalidated by a process of conditional deletion and the genome of which comprises a gene selected among reporter gene, market gene and gene encoding a protein of interest, inserted at the place of the DNA fragment deleted by said process of conditional deletion, said method comprises the steps of:

(i) preparation [Preparation] of a DNA molecule according to [claims 1 to 23] claim 1 wherein sequence A or sequence B is coding at least for part of the DNA fragment of interest to be invalidated and sequence B or sequence A is coding at least for a reporter gene;

(ii) obtention [Obtention] of a transgenic cell genetically modified by the targeted insertion by homologous recombination at the place of said DNA sequence of interest, of a DNA molecule prepared at step (i);

(iii) contacting [Contacting] said DNA molecule with at least one site specific recombinase [specific of] targeting sequence SSRTS L1 and one site specific recombinase [specific of] targeting sequence SSRTS L2;

(iv) inversion [Inversion] of sequences A and B or sequence A or sequence B by recombination catalyzed by said recombinase at either SSRTS L1 or SSRTS L2 sequences; and

(v) excision [Excision] of a DNA sequence by recombination catalyzed by said recombinase at SSRTS L2 or SSRTS L1 respectively, these SSRTS L2 or SSRTS L1 sequences being now present in direct orientation following to the inversion of step (iii), and being to recombine with one another.

36. (Amended) The method [Method] of claim 35, wherein the order of sequences in said DNA molecule is 5'-L1-sequence A-L2-sequence B-L1-L2-3' and wherein a sequence of homology with the DNA sequence of interest are present at both extremities of said DNA molecule and wherein, the DNA fragment excised in step (v) comprises sequence A.

37. (Amended) The method [Method] of claim 35, wherein the order of sequences in said DNA molecule is 5'-L1-L2- sequence A-sequence B-L1-L2-3' and wherein a sequence of homology with the DNA sequence of interest are present at both extremities of said DNA molecule.

38. (Amended) The method [Method] of claim 35 wherein the order of sequences in said DNA molecule is 5'-L1-L2-sequence A-L1-sequence B-L2-3' and wherein a sequence of homologis with the DNA sequence of interest are present at both extremities of said DNA molecule.

39. A method [Method] to perform site-specific recombination mediated cassette exchange (RMCE), said method comprising the steps of:

(i) [Preparation] preparation of a first DNA molecule comprising a first DNA sequence of interest flanked by incompatible SSRTS L1 and L2 in an opposite direction, obtainable by the method of [claims] claim 33 [to 38];

(ii) [Preparation] preparation of a second DNA molecule comprising a second DNA sequence of interest flanked by the same incompatible SSRTS L1 and L2 as in step (i) in an opposite direction, by *in vitro* DNA cloning;

(iii) contacting said first and said second DNA molecule with at least one site specific recombinase [specific of said] targeting sequence SSRTS L1 and one site specific recombinase [specific specific of said] targeting sequence SSRTS L2;

(iv) exchange [Exchange] by recombination catalysed by said recombinase of said first and said second DNA sequence of interest comprised in between the SSRTS L1 and L2.

40. (Amended) The method [Method] according to claim 39 wherein said second DNA molecule of step (ii) is obtainable by the method of [claims] claim 33 [to 38].

41. (Amended) The method [Method] according to [claims] claim 33 [to 40] wherein the steps are made in a cell free system.

42. (Amended) The method [Method] according to claim 33 [claims 33 to 40] wherein the steps are made in [the] an isolated transgenic host cell [of claims 26 to 31] transformed by an isolated DNA molecule comprising at least a sequence A flanked by at least site specific recombinase targeting sequences (SSRTS) L1, and at least a sequence B flanked by at least site specific recombinase targeting seqnences (SSRTS) L2, said SSRTS L1 and SSRTS L2 being unable to recombine with one another, and wherein:

(i) sequences L1 are in opposite orientation, and

(ii) sequences L2 are in opposite orientation, and

(iii) the order of SSRTS sequences in said DNA molecule is 5'-L1-L2-L1-L2-3'.

43. (Amended) The method [Method] according to claim 42, further comprising the step of introducing into the cell a gene encoding the corresponding site-specific recombinase.

44. (Amended) The method [Method] according to claim 43, wherein the gene encoding said site-specific recombinase is contained in an expression vector.

45. (Amended) The method [Method] according to claim 43, wherein the gene encoding said site-specific recombinase is stably inserted into the genome of said cell.

46. (Amended) The method [Method] according to [claims] claim 33 [to 45], wherein either SSRTS L1 comprises the Lox P1 sequence and SSRTS L2 comprises the Lox 511 sequence, or SSRTS L1 comprises the Lox 511 sequence and SSRTS L2 comprises Lox P1 sequence, and wherein the corresponding site-specific recombinase is Cre or its material or synthetic variants.

49. (Amended) A non-human living [Living] organism[, except humans,] that comprises at least one transgenic cell obtainable by the method of [claims] claim 33 [to 46].

50. The non-human living [Living] organism of claim 49, wherein said organism is selected [in] from the group consisting of bacteria, yeast, Caenorhabditis elegans, Drosophila melanogaster, zebrafish, mice, rat, rabbit, hamster, Guinea pig, cow, pig, goat, sheep, horse, and primate.

51. The non-human living [Living] organism of claim 50, wherein said organism is a mouse.

52. The non-human living [Living] organism of claim 50, wherein the organism is a yeast.